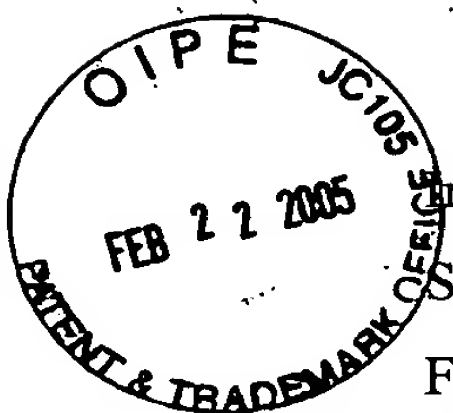


**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**



In re patent application of Alexander Vainstein et al.

Serial No. 09/914,146

Group Art Unit: 1638

Filed: January 29, 2002

Examiner: B. Koroma

For: **TRANSGENIC PLANTS AND METHOD FOR TRANSFORMING**

**DECLARATION**  
**under Rule 132**

Commissioner of Patents and Trademarks  
Washington, D.C. 20231

I, Alexander Vainstein, an Israeli citizen residing at 42/6 Hanassi Harishon St., Rehovot, Israel, hereby declare:

1. I am currently associate professor at the Faculty of Agricultural, Food and Environmental Quality Sciences of the Hebrew University of Jerusalem, Israel, and Head of the Hebrew University Graduate Biotechnology Program.
2. My list of publications is attached herewith as Annex "A". My fields of expertise include molecular biology, horticulture, flower breeding and plant transformation.
3. I am familiar with the above captioned application (hereinafter "*the application*"), and with the claims thereof. The invention relates to a method for changing the fragrance of a plant by modulating gene expression in the anthocyanin-biosynthetic pathway of the plant using an oligonucleotide molecule.
4. In general, the fragrance of an object is a function of both the relative amounts of various volatile molecules produced by the object as well as of the integration of the sensation by the one who smells. Thus, it is not an *increase* in fragrance which is disclosed in the application, but rather a *change* in fragrance, as a certain type of scent for one person may be perceived by another person as a different scent. Spraying a larger amount of one type of perfume results in an *increase* in fragrance, while spraying several types of perfume at the same time results in a *change* of fragrance. Therefore, in defining the invention, it is more correct to speak of a change in fragrance rather than an increase.

5. The above concept is well known in the scientific literature. For example, Araneda, R. C. et al (2000) *The Molecular Receptive Range of an Odorant Receptor*, Nature Neuroscience 3(12): 1248-1255 (Annex B), states in the abstract: "*An odor perception is the brain's interpretation of the activation pattern of many peripheral sensory neurons that are differentially sensitive to a wide variety of odors.*" Vainstein, A. et al (2001) *Floral Fragrance. New Inroads into an Old Commodity*, Plant Physiol. 127:1383-1389 (Annex C), states on page 1383 (left column): "*Flower scent is a composite character that is determined by a complex mixture of low-molecular-weight volatile molecules.*", and on page 1384 (right column): "*...the very same fragrance compounds can be present in flowers that are perceived by humans as having quite different scents*".

6. Although the application includes a description of experiments carried out using an antisense molecule to suppress expression of the *fht* gene, it is well known that post-transcriptional gene silencing (PTGS) may be carried out using a variety of strategies. One example of an alternate strategy is the use of RNA interference (RNAi). The use of this and other techniques in plants is described, for example, in Gura, T. (2000) *A silence that speaks volumes*, Nature 404:804-808 (Annex D) (see in particular the paragraph bridging pages 804-805, and page 807 last two paragraphs), and in Wang, E. and Wagner, G.J. (2003) *Elucidation of the functions of genes central to diterpene metabolism in tobacco trichomes using posttranscriptional gene silencing*, Planta 216:686-691 (Annex E).

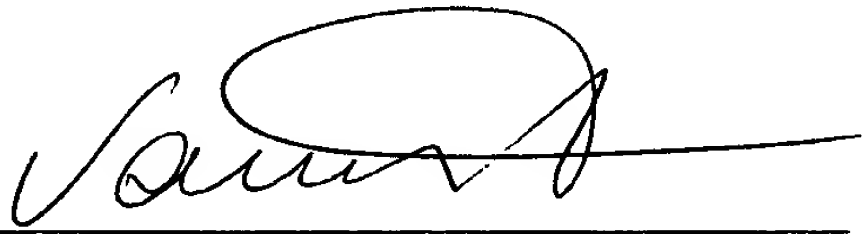
7. Furthermore, the invention is not limited to modulating the expression of the *fht* gene in the anthocyanin-biosynthetic pathway. The skilled man of the art will understand that other genes in this pathway may also be modulated to achieve the same result – changing the fragrance of the plant. As is stated in the application (page 8, lines 12-14): "*Approach to olfactory enhancement of natural fragrance (flower fragrance in particular) via diverting metabolic flow from anthocyanins to the production of benzoic acid derivatives (fragrance compounds) is demonstrated in carnation*". It is the control of the metabolic flow which determines the fragrance of the plant.

8. The invention described in the application is revolutionary in linking between plant fragrance and the anthocyanin-biosynthetic pathway. Prior to the invention, scientists in the field related to the anthocyanin-biosynthetic pathway

only in the context of flower color, and not in the context of fragrance. For example, see Gutterson, N. (1995) *Anthocyanin Biosynthetic Genes and Their Application to Flower Color Modification through Sense Suppression*, HortScience 30(5):964-966 (Annex F), and Holton, T.A. and Cornish, E.C. (1995) *Genetics and Biochemistry of Anthocyanin Biosynthesis*, The Plant Cell 7:1071-1083 (Annex G).

9. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 30/1/05



Prof. Alexander Vainstein

January 2005

## **CURRICULUM VITAE**

### ***ALEXANDER VAINSTEIN***

The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture  
Faculty of Agricultural, Food and Environmental Quality Sciences  
The Hebrew University of Jerusalem  
Rehovot 76100, Israel

#### Personal Details

Date and Place of Birth: February 24, 1957, Tbilisi, USSR  
Date of Immigration: December 4, 1977  
ID number: 017472143  
Citizenship: Israeli  
Marital Status: Married, two children  
IDF Military Service: 1983-1986  
Permanent address: Hanasi Harishon 42/6, Rehovot 76302, Israel  
Phone: 08-9489082 (work); 08-9475517 (home)  
Fax: 08-9489091  
Email: vain@agri.huji.ac.il

#### Education

B.Sc. - State University of Tbilisi, USSR, 1976  
Ph.D. - The Hebrew University of Jerusalem, Israel (Supervisor: Prof. A. Loyter), 1984  
Postdoctorate - University of California at Los Angeles, USA (Dr. E. Tobin's and Dr. J.P. Thornber's laboratories), supported by a BARD fellowship and a McKnight research award, 1986-1988

#### Appointments at the Hebrew University

1988-93     Lecturer, Department of Horticulture, Faculty of Agriculture, HUI  
1994-99     Senior Lecturer, Department of Horticulture, Faculty of Agriculture, HUI  
1999-present     Associate Professor, Department of Horticulture, Faculty of Agriculture, HUI

#### Additional Functions/Tasks at the HUI

1988-present     Member of the Genetics and Breeding Study Division, Faculty of Agriculture, HUI  
1990-present     Member of the Teaching Committee, Horticulture Division, Faculty of Agriculture, HUI  
1990-present     Member of the Otto Warburg Center for Biotechnology in Agriculture, Faculty of Agriculture, HUI  
1998-2001     Member of the Research Committee, Faculty of Agriculture, HUI  
2000-present     Member of the Steering Committee, The Otto Warburg Center for Biotechnology in Agriculture  
2000-03     Member of the Committee for Professional Advancement, HUI  
2001-02     Head of the Department of Horticulture, Faculty of Agriculture, HUI

2001-present	Head of the "Lehava" (Towards Higher Education at the HUI) Program
2001-present	Head of the Faculty of Agriculture Graduate Horticulture Program, HUI
2002-present	HUI representative to EPSO
2002-present	Head of the Hebrew University Graduate Biotechnology Program

#### Service in Other Academic and Research Institutions

1991	Member of the Committee for R & D Planning and Budgeting, Israeli Ministry of Agriculture, Ornamentals Section
1991	Member of the Planning Team, Israeli Ministry of Agriculture, Ornamentals Section
1993-present	Israel Science Foundation, Ministry of Science, BARD, DIARP, Ministry of Agriculture—review panel
1997-98	Sabbatical in Dr. J. Cohen's laboratory, USDA, Beltsville, MD, USA
2000-02	Israeli Society of Plant Sciences—Treasurer
2000-present	Member of the Committee for Professional Advancement, The Volcani Center, Israel
2000-present	Member of the Biotechnology Steering Committee, Israeli Ministry of Agriculture
2000-present	Member of the Steering Committee on Ornamentals, Israeli Ministry of Agriculture
2002-present	Israeli national representative to COST
2003-04	Sabbatical in Dr. V. Citovsky's laboratory, State University of New York, Stony Brook, NY, USA
2003-present	President of the Israeli Society of Plant Sciences

#### Other Academic/Professional Appointments and Awards Received

1989/90	Recipient of the Walter and Elise Haas Award
1991/92	Recipient of a Pretenure Glasgow Lectureship
1992	Member of the Organizing Committee for a meeting between scientists from the USSR and the Faculty of Agriculture, held at the Faculty of Agriculture, HUI
1992/93	Pretenure Glasgow Lectureship
1993	Recipient of the Golda Meir Fund Award
1993	Recipient of the Ellis Birk Fund Award
1993-99	Incumbent of the Cyril Rosenbaum Senior Lectureship in Horticulture
1994	Member of the Organizing Committee for an international conference on "Molecular Biology in Plant Breeding," held at the Faculty of Agriculture in honor of the 10 <sup>th</sup> anniversary of the Otto Warburg Center for Biotechnology in Agriculture
1995	Chairman of the Organizing Committee for the XVIII <sup>th</sup> EUCARPIA Symposium (Section Ornamentals) on "Ornamental Plant Improvement: Classical and Molecular Approaches," Tel Aviv, Israel
1998	Member of the Organizing Committee for the IX <sup>th</sup> International Congress on "Plant Tissue and Cell Culture," Jerusalem, Israel

- 1999 Recipient of the Agritech 99 and the Ministry of Agriculture Award for Excellence
- 1999-2000 Member of the Organizing Committee for the III<sup>rd</sup> International Symposium on "Rose Research and Cultivation," Herzlia, Israel
- 2000 Member of the Organizing Committee for an international Otto Warburg symposium on "Biotechnology for a Better Environment," held at the Faculty of Agriculture, HUI
- 2000 Member of the Organizing Committee for the DIARP Binational Workshop on "Advances in Handling, Transportation and Logistics of Ornamentals," Shefayim, Israel
- 2000-01 Member of the Organizing and Scientific committees for the II<sup>nd</sup> "Plants and Propagation Material" Conference, Israel
- 2001-03 Member of the Scientific Committee for the XXI<sup>st</sup> EUCARPIA Symposium (Section Ornamentals) on "Classical vs. Molecular Breeding of Ornamentals," Freising, Germany
- 2002-03 Member of the Organizing and Scientific committees for the III<sup>rd</sup> "Plants and Propagation Material" Conference, Israel
- 2003 Recipient of the Ellis Birk Fund Award
- 2003-present Member of the Organizing Committee for the XXII<sup>nd</sup> EUCARPIA Symposium (Section Ornamentals) on "Breeding for Beauty," to be held in 2006 in Italy

#### Member in professional societies

European Association for Research on Plant Breeding (EUCARPIA)  
 International Society for Horticultural Science (ISHS)  
 Israeli Society of Plant Sciences (IAPTCMB)

#### Service to Scholarly Publications

- 1991-present Associate Editor of Euphytica, International Journal of Plant Breeding, Kluwer Academic Publishers, the Netherlands
- 2000-03 Editorial board, Encyclopedia of Rose Science (Roberts, A., Editor in Chief), Elsevier Academic Press, UK
- 2002 Editor, "Breeding for Ornamentals: Classical and Molecular Approaches," Kluwer Academic Publishers, 392 pp., Dordrecht, the Netherlands.

## TEACHING AT THE HEBREW UNIVERSITY

### SUPERVISION OF STUDENTS (after appointment to associate professor)

#### M.Sc. students

1998-2000	Vered Canaan, Transgenic plants resistant to crown gall, degree completed. (#C24)
1999-2000	Shamir Tznuert, Transformation system for <i>Gypsophila</i> , degree completed <i>summa cum laude</i> .
1999-2001	Michal Lavy, Fragrance in carnation, co-supervisor D. Weiss, degree completed, <i>magna cum laude</i> . (#A46)
2000-02	Ofer Cohen, Regeneration and transformation in roses, degree completed.
2000-03	Eyal Capua, Triterpene saponin synthesis in <i>Gypsophila</i> , degree completed.
2000-03	Ran Amir, Identification and characterization of the locus <i>ita</i> from the plasmid RSF1010, degree completed.
2001-03	Chanan Himelfarb, Adaptation to salinity, co-supervisor A. Lers, degree completed.
2001-04	Sarit Neder, Use of <i>tachypleisin</i> I for improvement of rose vase life, co-supervisor N. Golob, thesis completed.
2002-04	Coby Buchs Dorf, Delay of flowering in leaf crops, co-supervisor A. Samach, degree completed.
2002-04	Efrat Izhaki, Rose genomics, degree completed.
2003-present	Shely Zafriar, Cytoskeleton changes in <i>Narcissus</i> during development, co-supervisor E. Sadot.
2003-present	Moran Farhi, Generation of plant aroma metabolites in yeast, co-supervisor H. Abeliovich.
2003-present	Hanoch Glasner, The use of <i>knotted1</i> to delay senescence of leaves and to prolong vase life of flowers, co-supervisor N. Ori.
2004-present	Yosefa Bar Zvi, Transgenic tobacco with <i>tachypleisin</i> I, co-supervisor N. Golob.
2004-present	Orly Lavie, Identification and characterization of rose gene for phenylethyl alcohol production.

#### Ph.D. students

1995-2000	Michael Vishnevetsky, Carotenoid-associated proteins, degree completed. (#C22,A26,A29,A33A37A40)
1996-2000	Amir Zuker, Transgenic carnation plants with novel traits, degree completed. (#C7,C23,C25,C26,C28,C29,C31,A24,A27,A30,AA334,A38,A40,A42,A45,A46A49,A52)
1997-2003	Gidon Scovel, Genetic characterization of horticultural traits in carnation, degree completed. (#C18,C30,C29,A35,A41A42,A51)



1998-2003	Hagit Ben-Meir, Characterization of the flavonoid biosynthetic pathway in <i>Gypsophila</i> , degree completed. (#C23,XC5,C26,C28,C29C31,A35A38,A45)
1999-present	Inna Gutterman, Rose genomics, co-supervisor D. Weiss. (#A44,A47,A48,50,A53)
1999-present	Yael Leitner, Tomato proteins involved in the sequestration of hydrophobic molecules.
2000-present	Mery Yelin, Rose proteome, co-supervisor Z. Adam. (#A44)
2002-present	Michal Ben-Zvi, Metabolic flow within and between pathways for floral scent and color. (#C35)
2003-present	Alon Glick, Molecular markers for resistance to <i>Botrytis</i> in rose, co-supervisor S. Meir.

#### Associate researchers in the lab

1995-present	Marianna Ovadis, Ph.D., senior researcher (#C23,C24,C26,C30,A26,A29,A37,A40,A42,A48)
1995-present	Elena Schklerman, M.Sc., researcher (#C23,C25,C26,C29,A42,A45)
1999	Dogan Sakar, Ph.D., visiting professor, Turkey (#C24)
2000-2001	Eva Cosanova, visiting Ph.D. student, Departament de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain (#A49,A52,A54)
2000	Xinlu Chen, Ph.D., postdoctoral fellow, Rose tissue culture
2002	Qiaochun Wang, Ph.D., postdoctoral fellow, Rose transformation



## **COURSES TOUGHT**

### Undergraduate courses

Classical and molecular approaches in plant breeding (71021) (agri-courses-w\courses\71021)

Principles of growth, storage and transportation of ornamentals (71466)

Basics of molecular biology (71204) (agri-courses-w\courses\71204)

### Graduate courses

Biological membranes (71978) (agri-courses-w\courses\71978 Biological Membranes)

Molecular control of pigmentation (73510)

Seminar in horticulture (71188) (agri-courses-w\courses\71187-Horticulture Seminar)

January, 2005

**ALEXANDER VAINSTEIN**

The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture  
Faculty of Agricultural, Food and Environmental Quality Sciences  
The Hebrew University of Jerusalem  
Rehovot 76100, Israel

**LIST OF PUBLICATIONS**

Principal investigator (PI), Student (S), co-researcher (C), technician/laboratory assistant (T)

**Ph.D. thesis**

**Vainstein, A.** (1984)

Reconstituted Sendai virus envelopes as carriers for the introduction of biological material (proteins and DNA) into eukaryotic cells

Ph.D. thesis submitted to the senate of the Hebrew University of Jerusalem

Supervisor - Prof. A. Loyter.

**Books**

**Books edited**

Before appointment to associate professor:

1. **Vainstein, A.** and Weiss, D. (eds.) (1995) Ornamental plant improvement: classical and molecular approaches. Acta Hort. 420, 149 pp., Leiden, the Netherlands.

After appointment to associate professor:

2. **Vainstein, A.** (ed.) (2002) Breeding for ornamentals: classical and molecular approaches. Kluwer Academic Publishers, 392 pp., Dordrecht, the Netherlands.

**Chapters in collections**

Before appointment to associate professor:

1. **Vainstein, A.**, Atidia, J. and Loyter, A. (1981) Reconstituted Sendai virus envelopes as a biological carrier for microinjection of proteins and DNA molecules. In "Liposomes in Study of Drug Activity and Immunocompetent Cell Function" (Panaf, A. and Nicolau, C., eds.) Academic Press, NY, pp. 95-108.

2. **Vainstein, A.**, Razin, A., Graessman, A. and Loyter, A. (1983) Fusogenic reconstituted Sendai virus envelopes as a vehicle for introduction of DNA into viable mammalian cells. In "Methods in Enzymology" (Wu, R. and Moldave, K., eds.) Academic Press, NY, vol. 101, pp. 492-512.
3. Thornber, J.P., Peter, G.F., Chitnis, P.R., Nechushtai, R. and **Vainstein, A.** (1988) Some aspects of the molecular and cellular biology of the light-harvesting complex of photosystem II. In "Light Energy Transduction in Photosynthesis" (Stevens Jr., S.E. and Bryant, D.A., eds.) American Society of Plant Physiologists, Rockville, MD, pp. 137-154.
4. Thornber, J.P., Peter, G.F., Chitnis, P.R. and **Vainstein, A.** (1989) Molecular and cellular biology of the major light-harvesting pigment-protein (LHC IIb) of higher plants. In "Photosynthesis: Molecular Biology and Bioenergetics" (Singhal, G.S. et al., eds.) Springer-Verlag-Narosa Publishing House, New Dehli, pp. 373-387.
5. **Vainstein, A.**, Razin, A., Graessmann, A. and Loyter, A. (1989) Fusogenic reconstituted Sendai virus envelopes as a vehicle for introduction of DNA into viable mammalian cells. In "Methods in Enzymology" (Wu, R., Grossman, L. and Moldave, K., eds.) Academic Press, NY, vol: Recombinant DNA Methodology, pp. 633-652.
6. **Vainstein, A.**, Fisher, M. and Ziv, M. (1992) Plant regeneration from carnation petals as a source for genetic variation in vitro. Acta Hort. 314:39-45.
7. **Vainstein, A.**, Hillel, J., Lavi, U. and Tzuri, G. (1992) Genetic variation detected by DNA fingerprinting in flowers. Acta Hort. 314:345-351.
8. **Vainstein, A.** (1993) Recent technical and scientific developments for the identification of varieties and the definition of minimum distances. In "The Protection of New Plant Varieties" CIOPORA, Switzerland, pp. 11-21.
9. **Vainstein, A.**, Ben-Meir, H. and Zuker, A. (1993) DNA fingerprinting as a reliable tool for the identification and genetic analysis of ornamentals. In "Creating Genetic Variation in Ornamentals" (Sciva, T. and Mercuri, A., eds.) EUCARPIA, Italy, pp. 373-387.
10. Altman, A., Yaari, A., Pelah, D., Gal, A., Tzfira, T., Wang, W.X., Shoseyov, O., **Vainstein, A.** and Riov, J. (1995) *In vitro* organogenesis, transformation and expression of drought-related proteins in forest tree cultures. In "Current Issues in Plant Molecular and Cellular Biology" (Terzi, M. et al., eds.) Kluwer Academic Publishers, the Netherlands, pp. 87-94.
11. Libal-Weksler, Y., Vishnevetsky, M., Ovadis, M., Itzhaki, H. and **Vainstein, A.** (1995) Flower-specific carotenoid accumulation in chromoplasts: molecular control of carotenoid-associated proteins. Acta Hort. (**Vainstein, A.** and Weiss, D., eds.) 420:32-34.

12. **Vainstein, A.** (1995) Ornamental plant improvement: classical and molecular approaches *Chronica Hort.* 35:8-9.
13. **Vainstein, A.**, Ben-Meir, H., Zuker, A., Watad, A., Scovel, G., Ahroni, A. and Ovadis, M. (1995) Molecular markers and genetic transformation in the breeding of ornamentals. *Acta Hort.* (**Vainstein, A.** and Weiss, D., eds.) 420:65-67.
14. Tzfira, T., Ben-Meir, H., Yarnitzky, O., **Vainstein, A.** and Altman, A. (1996) Highly efficient transformation and regeneration of transgenic aspen plants through shoot-bud formation in root culture, and transformation of *Pinus halepensis*. In "Somatic Cell Genetics and Molecular Genetics of Trees" (Ahuja, R. et al., eds.) Kluwer Academic Press, the Netherlands, pp. 125-130.
15. Altman, A., Pelah, D., Gal, A., Tzfira, T., Yarnitzky, O., Shoseyov, O. and **Vainstein, A.** (1996) Toward water stress-tolerant poplar and pine trees: molecular biology, transformation and regeneration. In "Somatic Cell Genetics and Molecular Genetics of Trees" (Ahuja, R. et al., eds.) Kluwer Academic Press, the Netherlands, pp. 47-56.
16. Vinocur, B., Tzfira, T., Ziv, M., **Vainstein, A.** and Altman, A. (1997) Bud regeneration and growth from transgenic and non-transgenic aspen (*Populus tremula*) root explants. In "Biology of Root Formation and Development" (Altman, A. and Waisel, Y., eds.) Basic Life Sciences Series, Plenum Publishing Co., NY, pp. 217-219.
17. Zuker, A., Ahroni, A. and **Vainstein, A.** (1997) A highly efficient method for carnation transformation. *Acta Hort.* (Altman, A. and Ziv, M., eds.) 447:373-375.
18. Ben-Meir, H., Scovel, G., Ovadis, M. and **Vainstein, A.** (1997) Molecular markers in the breeding of ornamentals. *Acta Hort.* (Altman, A. and Ziv, M., eds.) 447:599-601.
19. Tzfira, T., Jensen, C.S., **Vainstein, A.** and Altman, A. (1997) Improved rooting ability and root-system performance in transgenic aspen plants. In "Biology of Root Formation and Development" (Altman, A. and Waisel, Y., eds.) Basic Life Sciences Series, Plenum Publishing Co., NY, pp. 181-186.
20. Jensen, C.S., Tzfira, T., **Vainstein, A.** and Altman, A. (1997) Direct regeneration and selection of *Populus tremula* L. transgenic shoots from *Agrobacterium tumefaciens*-transformed stem explants. In "Biology of Root Formation and Development" (Altman, A. and Waisel, Y., eds.) Basic Life Sciences Series, Plenum Publishing Co., NY, pp. 209-211.
21. Tzfira, T., Jensen, C.S., **Vainstein, A.** and Altman, A. (1997) Aspen transformation procedures: oncogenic *Agrobacterium rhizogenes* versus disarmed *A. tumefaciens*. *Acta Hort.* (Altman, A. and Ziv, M., eds.) 447:295-300.

22. Vishnevetsky, M., Ovadis, M., Libal-Weksler, Y., Itzhaki, H. and **Vainstein, A.** (1997) Molecular analyses of carotenoid-associated proteins from chromoplasts of *Cucumis sativus* corollas. *Acta Hort.* (Altman, A. and Ziv, M., eds.) 447:575-578.
23. Ovadis, M., Zuker, A., Ahroni, A., Tzfira, T., Itzhaki, H., Shklarman, E., Ben-Meir, H. and **Vainstein, A.** (1999) Application of an integrative system based on microprojectile bombardment and *Agrobacterium tumefaciens* to generate transgenic carnation plants with novel characteristics. In "Plant Biotechnology and *In Vitro* Biology" (Altman, A., Izhar, S. and Ziv, M., eds.) Kluwer Academic Press, the Netherlands, pp. 189-192.
24. Ovadis, M., Chernin, L., Tzfira, T., Canaan, V., Aharoni, A., Sakar, D. and **Vainstein, A.** (1999) Transformation of tobacco and aspen plants with the *ita* locus of an INCQ plasmid confers resistance to *Agrobacterium tumefaciens* infection. In "Plant Biotechnology and *In Vitro* Biology" (Altman, A., Izhar, S. and Ziv, M., eds.) Kluwer Academic Press, the Netherlands, pp. 533-536.
25. Zuker, A., Tzfira, T., Scovel, G., Shklarman, E., Ovadis, M., Itzhaki, H., Ben-Meir, H. and **Vainstein, A.** (1999) Transgenic carnation plants. *Focus* 5-6.

After appointment to associate professor:

26. Ovadis, M., Zuker, A., Ahroni, A., Tzfira, T., Itzhaki, H., Shklarman, E., Ben-Meir, H. and **Vainstein, A.** (2000) Highly efficient procedure for generating transgenic carnation with novel traits. *Acta Hort.* (Le Nard, M. and Cadic, A., eds.) 508:49-51.
27. Altman, A., Tzfira, T., Wang, W., Vinocur, B., Hazan, G. and **Vainstein, A.** (2000) Clonal stability, seasonal periodicity and transgenes: the lesson from long-term micropropagation of transgenic and non-transformed *Populus tremula* L. plants. *Acta Hort.* 530:429-436.
28. Zuker, A., Tzfira, T., Ahroni, A., Shklarman, E., Ovadis, M., Itzhaki, H., Ben-Meir, H. and **Vainstein, A.** (2001) Genetic engineering of carnation (*Dianthus caryophyllus*). In "Biotechnology in Agriculture and Forestry" (Bajaj, Y.P.S., ed.) Springer-Verlag, Berlin, vol. 48, pp. 70-83.
29. Zuker, A., Shklarman, E., Scovel, G., Ben-Meir, H., Ovadis, M., Neta-Sharir, I., Ben-Yephet, Y., Weiss, D., Watad, A. and **Vainstein, A.** (2001) Genetic engineering of agronomic and ornamental traits in carnation. *Acta Hort.* (Sorvari, S., Karhu, S., Kanervo, E. and Pihakaski, S., eds.) 560:91-94.
30. Scovel, G., Ovadis, M., Reuven, M., Ben-Yephet, Y. and **Vainstein, A.** (2001) Marker-assisted selection for resistance to *Fusarium oxysporum* in the greenhouse carnation. *Acta Hort.* (Van Huylenbroeck J., Van Bockstaele, E. and Debergh P., eds.) 552:151-156.

31. Ben-Meir, H., Zuker, A., Weiss, D. and **Vainstein, A.** (2002) Molecular control of floral pigmentation: anthocyanins. In "Breeding for Ornamentals: Classical and Molecular Approaches" (Vainstein, A., ed.) Kluwer Academic Press, the Netherlands, pp. 253-272.
32. **Vainstein, A.**, Zamir, D. and Weiss, D. (2003) Rose fragrance--from gene to function. In "Encyclopedia of Rose Science" (Roberts, A. et al., eds.) Elsevier Academic Press, London, UK, pp. 263-265.
33. **Vainstein, A.**, Lewinsohn, E., Adam, Z., Pichersky, E., Zamir, D. and Weiss, D. (2003) Rose fragrance: genomic approaches and metabolic engineering. *Acta Hort.* (Forkmann, G., Hauser, B. and Michaelis, S., eds.) 612:105-111.
34. **Vainstein, A.**, Lewinsohn, E. and Weiss, D. (2004) A genomics approach for identification of floral scent genes in rose. In "Biology of Floral Scent" (Dudareva, N. and Pichersky, E., eds.) CRC Press, Boca Raton, FL (in press).
35. Ben Zvi, M. and **Vainstein, A.** (2004) Carnation biotechnology. In "Economic Crop Biotechnology" (Davey, M. and Chong, E., eds.) Springer-Verlag, (in press).

## Articles

### Before appointment to associate professor:

1. Loyter, A., **Vainstein, A.**, Graessmann, M. and Graessmann, A. (1983) Fusion-mediated injection of SV40-DNA: introduction of SV40-DNA into tissue culture cells by the use of DNA-loaded reconstituted Sendai virus envelopes. *Exp. Cell Res.* 143:415-425.
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### **Electronic plant gene register**

#### Before appointment to associate professor:

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### **Papers in non-reviewed professional journals**

#### Before appointment to associate professor:

- I. **Vainstein, A.**, Tzuri, G., Lavi, U. and Hillel, J. (1990)  
DNA fingerprinting in flowers  
*Dapei Meida* (Hebrew) 10:26.
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- III. Sharon, D., **Vainstein, A.**, Hillel, J. and Lavi, U. (1991)  
Application of DNA fingerprints to subtropical fruit trees for identification and breeding

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- IV. **Vainstein, A.** (1993)  
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## Abstracts of papers presented at national and international meetings

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Zuker, A., Ahroni, A. and **Vainstein, A.** (1997) Efficient transformation and regeneration of carnation. Israeli Society for Plant Tissue Culture and Molecular Biology Meeting, Tel Aviv, Israel.

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**Vainstein, A.**, Vishnevetsky, M. and Ovadis, M. (1999) Rapid transcriptional control is a key to phytohormonally regulated chromoplastogenesis in floral tissues. VII<sup>th</sup> Annual Symposium on Current Topics in Plant Biochemistry, Physiology and Molecular Biology, Columbia, MO, USA.

**Vainstein, A.** and Zuker, A. (1999) Genetic engineering of ornamentals. First Symposium on Cut Flowers, Bet Dagan, Israel.

Scovel, G., Blumenfeld, T. and **Vainstein, A.** (1999) The development and potential use of DNA markers in the breeding of cut carnation varieties. Second Symposium on Plant Propagation, Bet Dagan, Israel.

After appointment to associate professor:

Zuker, A., Shklarman, E., Ben-Meir, H., Ovadis, M., Watad, A. and **Vainstein, A.** (2000) Genetic engineering of agronomic and ornamental traits in carnation. The 4<sup>th</sup> International Symposium on In Vitro Culture and Horticultural Breeding, Tampere, Finland.

**Vainstein, A.** (2000) Genetic engineering of ornamentals. DIARP binational workshop on "Advances in Handling, Transportation and Logistics of Ornamentals," Shefayim, Israel.

**Vainstein, A.** (2000) Genetic modification of ornamentals. Rose Conference 2000, Kazanlak, Bulgaria.

**Vainstein, A.** (2002) Molecular breeding of ornamentals. Third Symposium on Quality Enhancement of Plant Production Through Tissue Culture, Kosice, Slovakia.

**Vainstein, A.**, Adam, Z., Lewinsohn, E., Zamir, D., Pichersky, E. and Weiss, D. (2003) Floral fragrance: genomic approaches and metabolic engineering. 21<sup>st</sup> EUCARPIA Symposium (Section Ornamentals) on Classical vs. Molecular Breeding of Ornamentals, Freising, Germany.

**Vainstein, A.** (2004) Deciphering the biology of flower aroma. Symposium on Plant Molecular Biology Programs and Perspectives in an Enlarged Europe, Warsaw, Poland.

Vaidya, M., Lacorix, B., **Vainstein, A.**, Tzfira, T. and Citovsky, V. (2004) An assay for protein transport from *Agrobacterium* to the host plant cell. Plant Biology 2004, Florida, USA.

Dafny-Yelin, M.; Guterman, I., Menda, N., Weiss, D., Adam, Z. and **Vainstein A.** (2004) Flower proteome—changes in protein spectrum during the advanced stages of rose petal development Plant Biology 2004, Florida, USA.

**Vainstein, A.** (2004) Proteome versus transcriptome during plant development. COST Workshop on "Assessment of Performance: Physiological Health and (Epi-)Genetic Stability," Saanen, Switzerland.

Leitner-Dagan, Y., Shklarman, E., Elad, Y., Rav, D., Ovadis, M. and **Vainstein, A.** (2005) Plastid lipid-associated protein CHRC: its involvement in chromoplastogenesis and plant development. The 4<sup>th</sup> Congress of the Federation of Israel Societies for Experimental Biology, Eilat, Israel.

Ben Zvi-Moyal, M., Ovadis, M., Shklarman, E., Zuker, A. and **Vainstein, A.** (2005) Cross-talk in the phenylpropanoid pathway: metabolic flow within and between pathways for floral scent and color. The 4<sup>th</sup> Congress of the Federation of Israel Societies for Experimental Biology, Eilat, Israel.

**Vainstein, A.** (2005) The scent of a rose: genomic approaches and metabolic engineering. Israel BioResearch Convention, Tel Aviv, Israel.

**Vainstein, A.** (2005) The scent of a rose: genomic approaches and metabolic engineering of flower fragrance. The 4<sup>th</sup> Congress of the Federation of Israel Societies for Experimental Biology, Eilat, Israel.



## Lectures at conferences and other activities

### Before appointment to associate professor:

- 1981-82 Visiting researcher in the laboratory of Prof. Adolf Graessmann at the Institute of Molecular Biology, Free University of Berlin, Germany (supported by an EMBO fellowship)
- 1983 Visiting researcher in the laboratory of Prof. George Klein at the Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden (sponsored by an ICRET fellowship)
- 1989 Visiting researcher at the Institute for Horticultural Plant Breeding, Wageningen, the Netherlands
- 1990-present Invited lecturer at the Ministry of Agriculture and at departmental seminars held at the HUJ, Tel Aviv University, the Weizmann Institute and the Technion, Israel
- 1991 Frontiers of Biotechnology in Agriculture, satellite meeting of the 15<sup>th</sup> International Congress of Biochemistry, Sea of Galilee, Israel (invited lecture)
- 1991 Second International Symposium on Propagation of Ornamental Plants, Herzlia, Israel (invited lecture)
- 1992 Israeli Photosynthesis Meeting, Rehovot, Israel (invited lecture)
- 1992 CIOPORA VI<sup>th</sup> International Symposium on the Protection of Plant Breeders' Rights, Munich, Germany (invited plenary lecture)
- 1993 XVII<sup>th</sup> EUCARPIA Symposium on Creating Genetic Variation in Ornamentals, San Remo, Italy (invited plenary lecture)
- 1994 International Conference on Molecular Biology in Plant Breeding, Rehovot, Israel (invited lecture)
- 1995 Israeli Symposium on Plant Sciences, Haifa, Israel (invited lecture)
- 1995 CPA Annual Symposium, Murcia, Spain (invited plenary-session lecture)
- 1996 Third International Symposium on In Vitro Culture and Horticultural Breeding, Jerusalem, Israel (invited lecture)
- 1997 Invited lecturer at the University of Florida, Gainesville, FL, USA and at the USDA, Beltsville, MD, USA
- 1998 Fifteenth Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, MD, USA (invited lecture)

### After appointment to associate professor:

- 1999 Seventeenth Annual Symposium on Current Topics in Plant Biochemistry, Physiology and Molecular Biology, MO, USA (invited lecture)
- 1999 First Symposium on Cut Flowers, Bet Dagan, Israel (invited lecture)
- 1999 Second Symposium on Plant Propagation, Bet Dagan, Israel (invited lecture)
- 2000 Third International Symposium on Rose Research and Cultivation, Herzlia, Israel (invited lecture)
- 2000 The 4<sup>th</sup> International Symposium on In Vitro Culture and Horticultural Breeding, Tampere, Finland (invited lecture)



- 2000 DIARP binational workshop on "Advances in Handling, Transportation and Logistics of Ornamentals," Shefayim, Israel (invited lecture)
- 2000 Rose Conference 2000, Kazanlak, Bulgaria (invited plenary lecture)
- 2002 Third Symposium on Quality Enhancement of Plant Production Through Tissue Culture, Kosice, Slovakia (invited plenary lecture)
- 2002 The 3<sup>rd</sup> Congress of the Federation of Israel Societies for Experimental Biology, Eilat, Israel (invited lecture)
- 2003 21<sup>st</sup> EUCARPIA Symposium (Section Ornamentals) on Classical vs. Molecular Breeding of Ornamentals, Freising, Germany (invited plenary lecture)
- 2003 Symposium on Plant Molecular Biology Programs and Perspectives in an Enlarged Europe, Warsaw, Poland (invited plenary lecture)
- 2004 Invited lecturer at the State University of New York, Stony Brook, NY, USA and at Purdue University, West Lafayette, IN, USA
- 2004 COST Workshop on "Assessment of Performance: Physiological Health and (Epi-)Genetic Stability," Saanen, Switzerland (invited lecture)
- 2005 The 4<sup>th</sup> Congress of the Federation of Israel Societies for Experimental Biology, Eilat, Israel (invited lecture)

**PATENTS**

**Vainstein, A.**, Vishnevetsky, M., Ovadis, M. and Itzhaki, H. (2003)  
Carotenoid-associated proteins useful for high carotenoid accumulation and production  
in plants and other organisms.  
United States Patent US 6,551,793.

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## RESEARCH ACTIVITIES

### General

My research involves various aspects of flower breeding, with an emphasis on the use of molecular techniques. In addition to the genetic engineering of ornamentals and the establishment of a molecular marker system for ornamentals, my research focuses on delineating basic molecular mechanisms of tissue-specific pigmentation. The overall goal of these studies is the breeding of state-of-the-art transgenic flowers, by harnessing novel genes that can be expressed and delivered to specific compartments within the cell at the desired developmental stage of the plant.

### Molecular flower breeding

This particular research is aimed at developing and employing genetic engineering approaches. We have developed a unique and efficient transformation procedure for carnation and more recently, for gypsophila (23,32,34,36,40; Pat.2). The main features of this carnation transformation procedure, which has been fully characterized, are its efficiency (ca. 2 transgenes per 10 explants) and suitability to numerous cultivars (40). It should be noted that low transformation efficiency is a very serious bottleneck in the creation of plants, carnations in particular, with novel traits of interest, since a large number of transgenes must be generated and screened to enable selection of the target genotype. We have used the established carnation transformation procedure to generate carnations with novel traits:

- 1) An antisense approach was employed to block the anthocyanin biosynthetic pathway, using the flavanone 3-hydroxylase (*FHT*) gene cloned from carnation; transgenic carnations were generated in an array of colors from a highly commercially successful monochromatic variety (66,68-70; Pat.2). Detailed characterization of the transgenes revealed that a complete blockage in anthocyanin production by *FHT*-antisense yields enhanced metabolic flow in the direction of benzoic acid derivatives and strong enhancement of fragrance (Pat.2). Two years of greenhouse testing have revealed that the transgenic lines are true-to-type and that the traits of interest (color, fragrance) are stable (70; Pat.2).
- 2) The *rolC* gene from *Agrobacterium rhizogenes*, driven by a CaMV 35S promoter, was harnessed to generate carnation plants with improved performance: transgenic lines exhibited dramatically improved rooting ability and production yield (in terms of both number of stem cuttings and number of flowering stalks per mother plant). Moreover,

these traits were stable following 2 years of greenhouse tests (66,68-70). Interestingly, in carnation *rolC* did not lead to the development of wrinkled leaves, compact form or low growth rate, or to any of the other highly negative traits often ascribed to *rol* genes. The effect of 35S:*rolC* in carnation is hence very similar to that of *rolC* driven by its native promoter in aspen, the latter having been quantitatively and qualitatively characterized by us (38,41,66,68-70). Note that the aspen study was made possible by our development of two alternative, highly efficient transformation systems (25,29).

Molecular genetic markers have been instrumental in advancing breeding programs, especially when genes of interest are not characterized and the genetic transformation approach is therefore not applicable. In the past we have used various DNA markers for cultivar identification, to establish genetic relatedness, and for evolutionary studies (20,21,51,52). Currently, we are using these markers to monitor loci linked to important horticultural traits in carnation, thus enabling early and effective selection in breeding programs (37,56,61). To this end, a carnation full-sib family segregating for flower type was generated and characterized. Using random decamer primers (RAPD), we identified a polymorphic DNA marker which is tightly linked to the recessive allele for “single” flower type (five petals per flower). A dominant mutation in this allele leads to an increased number of petals per flower (61). The RAPD marker was cloned and used to generate a restriction fragment length polymorphic (RFLP) marker that can discriminate between the semi-double and double flower phenotypes, and hence can be employed in marker-assisted breeding programs (37).

*Agrobacterium tumefaciens*, albeit a highly important tool for genetic engineering, causes crown gall, a disease that severely affects more than 1,000 plant species. In an attempt to develop an effective approach to controlling this disease, locus *ita*, responsible for the antitumorigenic activity of plasmid RSF1010, was located, characterized and harnessed to develop resistant transgenic plants: tobacco, tomato and aspen plants transgenic for locus *ita* were generated and shown, in both *in vitro* and greenhouse tests, to be resistant to crown gall disease (67; Pat.3). Taking into account the severity of this disease, the possibility of producing resistant transgenic ornamentals, e.g. rose, aster, etc. should be of high interest to the flower industry.

#### Chromoplast biogenesis in floral tissue

The objective of this study is to dissect the mechanism(s) regulating chromoplast biogenesis and carotenoid sequestration in floral tissue. Chromoplastogenesis in flowers and fruits involves the dramatic overaccumulation of carotenoids, sequestered with the aid of specific proteins into unique lipoprotein structures (39). We identified and cloned novel genes encoding two of these chromoplast-specific, carotenoid-associated proteins from cucumber flower corolla (CHRC and CHRD) (28,29; Pat.1). Based on studies of these proteins' pattern of accumulation as well as of carotenogenesis in flower corollas, a crucial role for phytohormones in general, gibberellin in particular, in chromoplastogenesis was revealed (31,35,39). We showed that CHRC and CHRD protein levels, together with carotenoid levels, are specifically up-regulated by GA<sub>3</sub> and down-regulated by ethylene and abscisic acid (ABA). The effects of GA<sub>3</sub> and ethylene on *CHRC* transcript levels were apparent after only 10-20 min. We showed that these major phytohormones – GA<sub>3</sub>, ABA and ethylene – affecting chromoplastogenesis in flowers, act as specific transcriptional regulators of *CHRC* expression. Furthermore, we found that GA<sub>3</sub> regulates *CHRC* expression in a primary fashion (28,31,35,39,42,43). This primary response was localized within the *CHRC* promoter to a 290-bp fragment which is both necessary and sufficient for activation (39,42,43; Pat.1). We also revealed that inhibition of carotenoid biosynthesis strongly down-regulates *CHRC* gene expression at the post-transcriptional and translational levels, thus demonstrating a tight link between the carotenoid biosynthetic pathway and that generating carotenoid-associated proteins (39,42). Based on the data, a working model has been presented wherein two major regulatory factors control carotenoid sequestration into unique structural compartments in the chromoplasts: 1) floral-specific isoprenoid-pathway-related transcriptional regulators of chromoplastogenesis; 2) post-transcriptional/translational regulators related to the amount/type of sequestered carotenoids (39,42). Delineating pathways regulating carotenoid-associated genes and elucidating the molecular interactions that govern the assembly of carotenoid-protein complexes should provide important clues as to the role of carotenoid sequestration in the plastid's developmental program and will undoubtedly also shed light on the molecular structure-functional composition of membrane-bound carotenoid biosynthetic enzymes. Detailed knowledge of these issues is, in fact, one of the major prerequisites

for success in applying molecular approaches to the generation of carotenoid cell factories.